Endpoint dilution using an antibody to the adenoviral hexon protein may be used to quantitate virus production or infection efficiency of target cells (Armentano et al. (1995) Hum. Gene Ther. 6:1343-53.

In addition to containing the DNA sequences encoding one or more transgenes, the chimeric adenoviral vectors of the invention may contain any expression control sequences such as a promoter or enhancer, a polyadenylation element, and any other regulatory elements that may be used to modulate or increase expression, all of which are operably linked in order to allow expression of the transgene. The use of any expression control sequences, or regulatory elements, which facilitate expression of the transgene is within the scope of the invention. Such sequences or elements may be capable of generating tissue-specific expression or be susceptible to induction by exogenous agents or stimuli.

Construction of Retroviral Vectors

The antigen presenting cells described herein can also be genetically modified with retroviral vectors produced recombinantly by procedures already taught in the art. For example, WO 94/29438 describes the construction of retroviral packaging plasmids and packaging cell lines. As is apparent to the skilled artisan, the retroviral vectors useful in the methods of this invention are capable of infecting the cells described herein. The techniques used to construct vectors, and transfect and infect cells are widely practiced in the art. Examples of retroviral vectors are those derived from murine, avian or primate retroviruses. Retroviral vectors based on the Moloney murine leukemia virus (MoMLV) are the most commonly used because of the availability of retroviral variants that efficiently infect human cells. Other suitable vectors include those based on the Gibbon Ape Leukemia Virus (GALV) or HIV.

The range of host cells that may be infected by a retrovirus or retroviral vector is determined by the viral envelope protein. The recombinant virus can be used to infect virtually any other cell type recognized by the env protein provided by the packaging cell, resulting in the integration of the viral genome in the

transduced cell and the stable production of the foreign gene product. In general, murine ecotropic env of MoMLV allows infection of rodent cells, whereas amphotropic env allows infection of rodent, avian and some primate cells, including human cells. Amphotropic packaging cell lines for use with MoMLV systems are known in the art and commercially available and include, but are not limited to, PA12 and PA317. Miller et al. (1985) Mol. Cell. Biol. **5**:431-437; Miller et al. (1986) Mol. Cell. Biol. **6**:2895-2902; and Danos et al. (1988) Proc. Natl. Acad. Sci. USA **85**:6460-6464. Xenotropic vector systems exist which also allow infection of human cells.

The host range of retroviral vectors has been altered by substituting the env protein of the base virus with that of a second virus. The resulting, "pseudotyped", virus has the host range of the virus donating the envelope protein and expressed by the packaging cell line. Recently, the G-glycoprotein from vesicular stomatitis virus (VSV-G) has been substituted for the MoMLV env protein. Burns et al. (1993) Proc. Natl. Acad. Sci USA **90**:8033-8037; and PCT patent application WO 92/14829. Since infection is not dependent on a specific receptor, VSV-G pseudotyped vectors have a broad host range.

Usually, the vectors will contain at least two heterologous genes or gene sequences: (i) the therapeutic gene to be transferred; and (ii) a marker gene that enables tracking of infected cells.

The viral vector can be modified to incorporate chimeric envelope proteins or nonviral membrane proteins into retroviral particles to improve particle stability and expand the host range or to permit cell type-specific targeting during infection. The production of retroviral vectors that have altered host range is taught, for example, in WO 92/14829 and WO 93/14188. Retroviral vectors that can target specific cell types *in vivo* are also taught, for example, in Kasahara et al. (1994) Science **266**:1373-1376. Kasahara et al. describe the construction of a Moloney leukemia virus (MoMLV) having a chimeric envelope protein consisting of human erythropoietin (EPO) fused with the viral envelope protein. This hybrid virus shows tissue tropism for human red blood progenitor cells that bear the

receptor for EPO, and is therefore useful in gene therapy of sickle cell anemia and thalassemia. Retroviral vectors capable of specifically targeting infection of cells are preferred for *in vivo* gene therapy.

The vector construct is introduced into a packaging cell line which will generate infectious virions. Packaging cell lines capable of generating high titers of replication-defective recombinant viruses are known in the art, see for example, WO 94/29438. Viral particles are harvested from the cell supernatant and purified for *in vivo* infection using methods known in the art such as by filtration of supernatants 48 hours post transfection. The viral titer is determined by infection of a constant number of appropriate cells (depending on the retrovirus) with titrations of viral supernatants. The transduction efficiency can be assayed 48 hours later by a variety of methods, including Southern blotting.

After viral transduction, the presence of the viral vector in the transduced cells or their progeny can be verified such as by PCR. PCR can be performed to detect the marker gene or other virally transduced sequences. Generally, periodic blood samples are taken and PCR conveniently performed using e.g. NeoR probes if the NeoR gene is used as marker. The presence of virally transduced sequences in bone marrow cells or mature hematopoietic cells is evidence of successful reconstitution by the transduced cells. PCR techniques and reagents are well known in the art, See, generally, PCR PROTOCOLS, A GUIDE TO METHODS AND APPLICATIONS. Innis, Gelfand, Sninsky & White, eds. (Academic Press, Inc., San Diego, 1990) and commercially available (Perkin-Elmer).

In vitro/ex vivo, exposure of human DCs to adenovirus (Ad) vector at a multiplicity of infection (MOI) of 500 for 16-24 h in a minimal volume of serum-free medium reliably gives rise to transgene expression in 90-100% of DCs. The efficiency of transduction of DCs or other APCs can be assessed by immunofluorescence using fluorescent antibodies specific for the tumor antigen being expressed (Kim et al., 1997). Alternatively, the antibodies can be conjugated to an enzyme (e.g. HRP) giving rise to a colored product upon reaction

with the substrate. The actual amount of antigen being expressed by the APCs can be evaluated by ELISA.

Transduced APCs can subsequently be administered to the host via an intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery.

In vivo transduction of DCs, or other APCs, can potentially be accomplished by administration of Ad (or other viral vectors) via different routes including intravenous, intramuscular, intranasal, intraperitoneal or cutaneous delivery. The preferred method is cutaneous delivery of Ad vector at multiple sites using a total dose of approximately $1x10^{10}$-$1x\ 10^{12}$ i.u. Levels of *in vivo* transduction can be roughly assessed by co-staining with antibodies directed against APC marker(s) and the TAA being expressed. The staining procedure can be carried out on biopsy samples from the site of administration or on cells from draining lymph nodes or other organs where APCs (in particular DCs) may have migrated. The amount of TAA being expressed at the site of injection or in other organs where transduced APCs may have migrated can be evaluated by ELISA on tissue homogenates.

Although viral gene delivery is more efficient, DCs can also be transduced *in vitro/ex vivo* by non-viral gene delivery methods such as electroporation, calcium phosphate precipitation or cationic lipid/plasmid DNA complexes. Transduced APCs can subsequently be administered to the host via an intravenous, subcutaneous, intranasal, intramuscular or intraperitoneal route of delivery.

In vivo transduction of DCs, or other APCs, can potentially be accomplished by administration of cationic lipid/plasmid DNA complexes delivered via the intravenous, intramuscular, intranasal, intraperitoneal or cutaneous route of administration. Gene gun delivery or injection of naked plasmid DNA into the skin also leads to transduction of DCs. Intramuscular delivery of plasmid DNA may also be used for immunization.

The transduction efficiency and levels of transgene expression can be assessed as described above for viral vectors.

Expansion of Immune Effector Cells

The present invention makes use of these APCs to stimulate production of an enriched population of antigen-specific immune effector cells. The antigen-specific immune effector cells are expanded at the expense of the APCs, which die in the culture. The process by which naïve immune effector cells become educated by other cells is described essentially in Coulie (1997) Molec. Med. Today 3:261-268.

In a preferred embodiment, the antigen-specific immune effector cells are CTLs. In one aspect, the cytotoxic T cells are polyclonal T cells isolated from a site of cytotoxic T cell infiltration from a subject. Alternatively, such cells may be isolated from a site of cytotoxic T cell infiltration from two or more subjects or human patients, in which the subjects share an MHC halotype. In another embodiment, the CTLs may be two or more cytotoxic T cell lines. In yet another embodiment, the CTLs may be any combination of the foregoing.

In a further aspect of the invention, the site of cytotoxic T cell infiltration is a tumor. The tumors from which cells or cell lines are obtained can be the same type of tumor in different individuals with a shared MHC halotype or different types of tumors from different subjects who share an MHC haplotype.

The APCs prepared as described above are mixed with naïve immune effector cells. Preferably, the cells may be cultured in the presence of a cytokine, for example IL2. Because dendritic cells secrete potent immunostimulatory cytokines, such as IL2, it may not be necessary to add supplemental cytokines during the first and successive rounds of expansion. In any event, the culture conditions are such that the antigen-specific immune effector cells expand (*i.e.* proliferate) at a much higher rate than the APCs. Multiple infusions of APCs and optional cytokines can be performed to further expand the population of antigen-specific cells.

In one embodiment, the immune effector cells are T cells and are specific for tumor-specific antigens which are presented by the APCs.

Compositions

This invention also provides compositions containing any of the above-mentioned proteins, muteins, polypeptides, nucleic acid molecules, vectors, cells antibodies and fragments thereof, and an acceptable solid or liquid carrier. When the compositions are used pharmaceutically, they are combined with a "pharmaceutically acceptable carrier" for diagnostic and therapeutic use. These compositions also can be used for the preparation of medicaments for the diagnosis and treatment of diseases such as cancer.

Tumor Protection in Animal Models

Applicants are the first to establish that, based on the animal models described below, prevaccination with the compositions of this invention will prevent or delay onset of disease.

The murine B16 melanoma model was used. In this model, C57BL/6 mice were immunized with bone marrow-derived DCs transduced with an Ad vector encoding either human gp100 (Ad/hugp100) or mouse gp100 (Ad/mgp100). Mice immunized against heterologous human gp100 developed a protective immune response and were resistant to a lethal subcutaneous challenge of B16 melanoma cells (syngeneic tumor cell line that expresses gp100). In contrast, mice immunized with homologous mouse gp100 failed to mount a protective immune response against B16 melanoma cells and developed tumors at the site of B16 cell injection. This finding illustrates the difficulty in breaking tolerance against a self antigen (mouse gp100). The corresponding heterologous antigen from a different species (human gp100), however, is likely to contain several Class I and Class II-associated epitopes that will be recognized as foreign and elicit $CD8^+$ and $CD4^+$ T cell responses, respectively. The induction of cross-reactive CTLs that recognize

both the heterologous and homologous self-antigen can then lead to lysis of host tumor cells.

Unfortunately, this type of animal model cannot be used to test the efficacy of modified or heterologous tumor antigens being considered for use in humans since mice and humans recognize different epitopes, primarily as a result of differences in their MHC molecules. It may be possible, however, to use the allogeneic human peripheral blood lymphocyte - severe combined immunodeficiency mouse (Hu-PBL-SCID) model. SCID mice lack mature B and T lymphocytes and can be reconstituted with human PBLs. It may be possible to immunize such mice with test antigen to induce a response in adoptively transferred human PBLs and evaluate protection against challenge with a human tumor cell line (Mosier et al., 1988; Parney et al., 1997; Albert et al., 1997).

Another possibility is immunization of HLA-A2.1 transgenic mice to reproduce the immune reactivity of HLA-A2 individuals.

Adoptive Immunotherapy and Vaccines

The expanded populations of antigen-specific immune effector cells of the present invention find use in adoptive immunotherapy regimes and as vaccines.

Adoptive immunotherapy methods involve, in one aspect, administering to a subject a substantially pure population of educated, antigen-specific immune effector cells made by culturing naïve immune effector cells with APCs as described above. Preferably, the APCs are dendritic cells.

In one embodiment, the adoptive immunotherapy methods described herein are autologous. In this case, the APCs are made using parental cells isolated from a single subject. The expanded population also employs T cells isolated from that subject. Finally, the expanded population of antigen-specific cells is administered to the same patient.

In another embodiment, the adoptive immunotherapy methods are allogeneic. Here, cells from two or more patients are used to generate the APCs, and stimulate production of the immune effector cells. For instance, cells from

other healthy or diseased subjects can be used to generate antigen-specific cells in instances where it is not possible to obtain autologous T cells and/or dendritic cells from the subject providing the biopsy. The expanded population can be administered to any one of the subjects from whom cells were isolated, or to another subject entirely.

In a further embodiment, APCs or immune effector cells are administered with an effective amount of a stimulatory cytokine, such as IL-2 or a co-stimulatory molecule.

The agents identified herein as effective for their intended purpose can be administered to subjects or individuals susceptible to or at risk of developing a disease, such as cancer. When the agent is administered to a subject such as a mouse, a rat or a human patient, the agent can be added to a pharmaceutically acceptable carrier and systemically or topically administered to the subject. To determine patients that can be beneficially treated, a tumor regression can be assayed. Therapeutic amounts can be empirically determined and will vary with the pathology being treated, the subject being treated and the efficacy and toxicity of the therapy. When delivered to an animal, the method is useful to further confirm efficacy of the agent. As an example of an animal model, groups of nude mice (Balb/c NCR nu/nu female, Simonsen, Gilroy, CA) are each subcutaneously inoculated with about 10^5 to about 10^9 hyperproliferative, cancer or target cells as defined herein. When the tumor is established, the agent is administered, for example, by subcutaneous injection around the tumor. Tumor measurements to determine reduction of tumor size are made in two dimensions using venier calipers twice a week. Other animal models may also be employed as appropriate.

Administration *in vivo* can be effected in one dose, continuously or intermittently throughout the course of treatment. Methods of determining the most effective means and dosage of administration are well known to those of skill in the art and will vary with the composition used for therapy, the purpose of the therapy, the target cell being treated, and the subject being treated. Single or

multiple administrations can be carried out with the dose level and pattern being selected by the treating physician. Suitable dosage formulations and methods of administering the agents can be found below.

The agents and compositions of the present invention can be used in the manufacture of medicaments and for the treatment of humans and other animals by administration in accordance with conventional procedures, such as an active ingredient in pharmaceutical compositions.

More particularly, an agent of the present invention also referred to herein as the active ingredient, may be administered for therapy by any suitable route including nasal, topical (including transdermal, aerosol, buccal and sublingual), parenteral (including subcutaneous, intramuscular, intravenous and intradermal) and pulmonary. It will also be appreciated that the preferred route will vary with the condition and age of the recipient, and the disease being treated.

The compositions and methods described herein are particularly useful in providing or inducing a prophylactic immune response in an animal. Animals in a pre-disease state or in a disease free interval, i.e., having or pre-disposed to a condition subject to immune surveillance, are most suitably treated by the methods and compositions described herein. Such conditions involve the activation of an immune response in a diseased state or period.

Example

Dendritic cells were derived from peripheral blood of a HLA-A2+ human donor using standard GM-CSF/IL-4 culture technique. After six days, the cells were infected with an adenovirus (serotype 2) construct encoding human gp 100. At T=7 days, they were restimulated with 5 x 10^5 infected autologous DCs (from frozen stocks) and given 50 U/ml rhIL-2. At T=14 days, the cultures were treated with leucyl-leucyl-methyl ester in order to eliminate NK cell activity. Immediately after treatment, the cells were washed thoroughly and replated along with 1 x 10^7 mitomycin C-treated autologous PBMC as feeders. At T=21 days,

cells were split and replated at 5 x 10^5 cells/ml in Iscoves/10% human AB serum/1000U rhIL-2/ml. At T=26 days, the CTLs raised against gp100 were tested in ^{51}Cr-release assay using peptide pulsed T2 cells as targets. Effector CTLs were TIL 1520 which specifically recognize an HLA-A-A2 restricted epitope of the wild-type human gp100 protein. Figure 5 shows the results of this assay. There is epitope-specific recognition that was not present prior to education of the T cells with the infected DCs.

It is to be understood that while the invention has been described in conjunction with the above embodiments, that the foregoing description and the following examples are intended to illustrate and not limit the scope of the invention. For example, any of the above-noted compositions and/or methods can be combined with known therapies or compositions. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

CLAIMS

1. A method of inducing a prophylactic immune response to a self-antigen in a subject, comprising administering to the subject an effective amount of the antigen or an altered form of the antigen.

2. The method of claim 1, wherein the antigen is administered as a polynucleotide coding for the self-antigen.

3. The method of claim 2, wherein the polynucleotide is delivered as naked DNA.

4. The method of claim 2, wherein the polynucleotide is delivered in a gene delivery vehicle.

5. The method of claim 1, further comprising administering an effective amount of an immunostimulatory agent to the subject.

6. The method of claim 5, wherein the immunostimulatory agent is administered as a polynucleotide coding for the immunostimulatory agent.

7. The method of claim 1, wherein the antigen is administered in an antigen presenting cell.

8. The method of claim 7, wherein the antigen presenting cell has been genetically modified by insertion of a polynucleotide coding for the antigen.

9. The method of claim 7, wherein the antigen presenting cell is a foster antigen presenting cell, a hybrid antigen presenting cell, or a pulsed antigen presenting cell.

10. The method of claim 7, wherein the antigen presenting cell is a dendritic cell.

11. The method of claim 7, further comprising administering an effective amount of an immunostimulatory agent to the subject.

12. The method of claim 11, wherein the immunostimulatory agent is administered as a polynucleotide coding for the immunostimulatory agent.

13. The method of claim 1 or 7, wherein the self-antigen is a tumor associated antigen (TAA).

14. A method of providing a prophylactic immune response to a self-antigen in a subject, comprising administering to the subject an effective amount of educated immune effector cells, educated to specifically recognize and lyse cells expressing the self-antigen or an altered form of the self-antigen.

15. The method of claim 14, wherein the immune effector cells have been produced by stimulating naïve immune effector cells with antigen presenting cells that present the antigen or an altered self-antigen to the naïve immune effector cells.

16. The method of claim 14, wherein the educated immune effector cells are produced *ex vivo*.

17. The method of claim 14, wherein the educated immune effector cells are produced *in vivo*.

18. The method of claim 14, further comprising administering an effective amount of an immunostimulatory agent.

19. The method of claim 18, wherein the immunostimulatory agent is administered as a polynucleotide coding for the immunostimulatory agent.

20. The method of claim 1 or 14, wherein the subject is characterized as being in a disease-free state but genetically predisposed to a condition subject to immune surveillance.

21. The method of claim 20, wherein the condition is associated with the presence of the HER-2/neu gene in the subject.

22. The method of claim 1 or 14, wherein the subject is characterized as being in a disease free interval of a condition subject to immune surveillance.

23. The method of claim 22, wherein the condition is melanoma.

FIG. 1A
FIG. 1B

FIG. 1

```
HUMAN     1  MDLVLKRCLL HLAVIGALLA VGATKVPRNQ DWLGVSRQLR TKAWNRQLYP
MOUSE        --*G-QR-SF- PLY-LSA--- ---LEGS--- -----P---V --T------

          51 EWTEAQRLDC WRGGQVSLKV SNDGPTLIGA NASFSIALNF PGSQKVLPDG
             ----V-GSN- ---------R- I------I-- ---------H- ----------

         101 QVIWVNNTII NGSQVWGGQP VYPQETDDAC IFPDGGPCPS GSWSQKRSFV
             ----A----- ---------- -----P---- V--------- --PKPP-----

         151 YVWKTWGQYW QVLGGPVSGL SIGTGRAMLG THTMEVTVYH RRGSRSYVPL
             --------K-- ---------R- --A--H-K-- ---------- ----Q-----

         201 AHSSSAFTIT DQVPFSVSVS QLRALDGGNK HFLRNQPLTF ALQLHDPSGY
             --A--T----- ---------- --Q----ET- -----H--I- ----------

         251 LAEADLSYTW DFGDSSGTLI SRALVVTHTY LEPGPVTAQV VLQAAIPLTS
             ---------- ----GT---- ----D----- --S-S----- -------V-
```

```
301  CGSSPVPGTT DGHRPTAEAP NTTAGQVPTT EVVGTTPGQA PTAEPSGTTS
     ---------- --YM------ G--SR-GT-- ---------- ----M --TQ-----V

351  VQVPTTEVIS TAPVQMPTAE STGMTPEKVP VSEVMGTTLA EMSTPEATGM
     --M-----TA -TSE--L-** ********** *-A-ID---- -V--T-G--T

401  TPAEVSIVVL SGTTAAQVTT TEWVETTARE LPIPEPEGPD ASSIMSTESI
     --T******P ----V--A-- --********* ********** --PLLP-Q-S

451  TGGLGPLLDG TATLRLVKRQ VPLDCVLYRY GSFSVTLDIV QGIESAEILQ
     ---IS----D -D-IM------ ---------- ----LA---- ----------

501  AVPSGEGDAF ELTVSCQGGL PKEACMEISS PGCQPPAQRL CQPVLPSPAC
     ---FS----- ---------- ------D--- ---------- --S-P---D-

551  QLVLHQILKG GSGTYCLNVS LADTNSLAVV STQLIMPGQE AGLGQVPLIV
     ------V--- ---------- ---A------A ----VV---D G----A--L-

601  GILLVLMAVV LASLIYRRRL MKQDFSVPQL PHSSHWLRL PRIFCSCPIG
     ------V--- -----H-H-- K--G*--S-M --G-T----- -PV-RARGL-

651  ENSPLLSGQQ VX
     ---------- --
```

FIG. 1B

FIG. 2A

FIG. 2A
FIG. 2B

FIG. 2

A

```
                10        20              30        40        50
mMART  ATGCCCCAAGAAGACATTCACTT------TGGTTATCCCAGGAAGGGGCACAGACGCTCC
hMART  ATGCCAAGAGAAGATGCTCACTTCATCTATGGTTACCCCAAGAAGGGGCACGGCCACTCT
                10        20        30        40        50        60

           60        70        80        90       100       110
mMART  TATGTCACTGCTGAAGAGGCCGCAGGGATCGGCATCCTGATCGTGGTCCTGGGGATTGCT
hMART  TACACCACGGCTGAAGAGGCCGCTGGGATCGGCATCCTGACAGTGATCCTGGGAGTCTTA
                70        80        90       100       110       120

          120       130       140       150       160       170
mMART  CTGCTTATCGGCTGCTGGTACTGTAGAAGACGAAGTGGATACAGAACCTTGATGGACAAA
hMART  CTGCTCATCGGCTGTTGGTATTGTAGAAGACGAAATGGATACAGAGCCTTGATGGATAAA
               130       140       150       160       170       180

          180       190       200       210       220       230
mMART  AGGCGTCATATTGGTATTCAAAAAACCTCAAGGGAAAGATGCTCATGTGAGAGCCCTGAT
hMART  AGTCTTCATGTTGGCACTCAATGTGCCTTAACAAGAAGATGCCCACAAGAAGGGTTTGAT
               190       200       210       220       230       240
```

```
                240        250        260        270        280        290
mMART    CACCAGGACAGCCGACTGTCTTCTCAAGAGAAATCCCATCAGCCCGTGGTTCCCAACGCT
         || | |||||||  | |||||  ||||||||||  |  | | ||||||||||| |||
hMART    CATCGGGACAGCAAAGTGTCTCTTCAAGAGAAAAACTGTGAACCTGTGGTTCCCAATGCT
                    250        260        270        280        290        300

                300        310                 320        330        340
mMART    CCGCCTGCCTATGAGAAGCTCTCT---------TCACCGCCACCTTATTCACCCTGA
         || ||||| |||||||| ||||||         ||||| |||||||||||||| | |
hMART    CCACCTGCTTATGAGAAACTCTCTGCAGAACAGTCACCACCACCTTATTCACCTTAA
                   310        320        330        340        350
```

B

```
                   10         20         30         40         50
mMART    MPQEDIHF--GYPRKGHRRSYVTAEEAAGIGILIVVLGIALLIGCWYCRRRSGYRTLMDK
         || || ||  ||| |||  || ||||||||||| | ||  ||||||||||| ||| ||||
hMART    MPREDAHFIYGYPKKGHGHSYTTAEEAAGIGILTVILGVLLLIGCWYCRRRNGYRALMDK
                 10         20         30         40         50         60

             60        70        80        90        100        110
mMART    RRHIGIQKTSRERCSCESPDHQDSRLSSQEKSHQPVVPNAPPAYEKLS---SPPPYSPX
           | | |     ||  |  || ||  | |||   ||||||||||||||   ||||||||
hMART    SLHVGTQCALTRRCPQEGFDHRDSKVSLQEKNCEPVVPNAPPAYEKLSAEQSPPPYSPX
                  70        80        90        100        110
```

FIG. 2B

FIG. 3A | FIG. 3B } FIG. 3

```
1     AGCAGACAGAGGACTCTCATTAAGGAAGG TGTCCTGTGCCTTGACCCTACAAGATGCCA
                                                            MetPro

120   ACGGCTGAAGAGGCCGCTGGGATCGGCATC CTGACAGTGATCCTGGGAGTCTTACTGCTC
23    ThrAlaGluGluAlaAlaGlyIleGlyIle LeuThrValIleLeuGlyValLeuLeuLeu

240   CATGTTGGCACTCAATGTGCCTTAACAAGA AGATGCCCACAAGAAGGGTTTGATCATCGG
63    HisValGlyThrGlnCysAlaLeuThrArg ArgCysProGlnGluGlyPheAspHisArg

360   GCTTATGAGAAACTCTCTGCAGAACAGTCA CCACCACCTTATTCACCTTAAGAGCCAGCG
103   AlaTyrGluLysLeuSerAlaGluGlnSer ProProProTyrSerPro

480   ATCTAATGTTCTCCTTTGGAATGGTGTAGG AAAAATGCAAGCCATCTCTAATAATAAGTC
600   TATTAAATTGGGAAAACTCCATCATAAAAT GTTGCAATGCATGATACTATCTGTGCCAGA
720   GGGGCCATCCAATTTCTCTTTACTTGAAAT TTGGCTAATAACAAACTAGTCAGGTTTTCG
840   GATACTTTTACAGGTTAAGACAAAGGGTTG ACTGGCCTATTTATCTGATCAAGAACATGT
960   CTATAGCTCTTTTTTTTGAGATGGAGTTT CGCTTTTGTTGCCCAGGCTGGAGTGCAATG
1080  CCTCCTGAGTAGCTGGGATTACAGGCGTGC GCCACTATGCCTGACTAATTTTGTAGTTTT
1200  TCTGCCCGCCTCAGCCTCCCAAAGTGCTGG AATTACAGGCGTGAGCCACCACGCCTGGCT
1320  AATGCTATTCTAACTAATGACAAGTATTTT CTACTAAACCAGAAATTGGTAGAAGGATTT
1440  TACCTATGGCAATTTAGCTCTCTTGGGTTC CCAAATCCCTCTCACAAGAATGTGCAGAAG
```

FIG. 3A

```
AGAGAAGATGCTCACTTCATCTATGGTTAC  CCCAAGAAGGGGCACGGCCACTCTTACACC  119
ArgGluAspAlaHisPheIleTyrGlyTyr  ProLysLysGlyHisGlyHisSerTyrThr  22

ATCGGCTGTTGGTATTGTAGAAGACGAAAT  GGATACAGAGCCTTGATGGATAAAAGTCTT  239
IleGlyCysTrpTyrCysArgArgArgAsn  GlyTyrArgAlaLeuMetAspLysSerLeu  62

GACAGCAAAGTGTCTCTTCAAGAGAAAAAC  TGTGAACCTGTGGTTCCCAATGCTCCACCT  359
AspSerLysValSerLeuGlnGluLysAsn  CysGluProValValProAsnAlaProPro  102

AGACACCTGAGACATGCTGAAATTATTTCT  CTCACACTTTTGCTTGAATTTAATACAGAC  479
                                                                118

AGTGTTAAAATTTTAGTAGGTCCGCTAGCA  GTACTAATCATGTGAGGAAATGATGAGAAA  599
GGTAATGTTAGTAAATCCATGGTGTTATTT  TCTGAGACACAGAATTCAAGTGGGTATTCT  719
AACCTTGACCGACATGAACTGTACACAGAA  TTGTTCCAGTACTATGGAGTGCTCACAAAG  839
CAGCAATGTCTCTTGTGCTCTAAAATTCT  ATTATACTACAATAATATATTGTAAAGATC  959
GCGCGATCTTGGCTCACCATAACCTCCGCC  TCCCAGGTTCAAGCAATTCTCCTGCCTTAG  1079
AGTAGAGACGGGGTTTCTCCATGTTGGTCA  GGCTGGTCTCAAACTCCTGACCTCAGGTGA  1199
GGATCCTATATCTTAGGTAAGACATATAAC  GCAGTCTAATTACATTTCACTTCAAGGCTC  1319
AAATAAGTAAAAGCTACTATGTACTGCCTT  AGTGCTGATGCCTGTGTACTGCCTTAAATG  1439
AAATCATAAAGGATCAGAGATTCTGAAAAA  AAAAAAAAAAAAAAAAAAAAAAAAAAAAA  1559
```

FIG. 3B

FIG. 4A
FIG. 4B
FIG. 4

MOUSE TRP2

```
1    MGLVGWGLLL GCLGCGILLR ARAQFPRVCM TLDGVLNKEC CPPLGPEATN
51   ICGFLEGRGQ CAEVQTDTRP WSGPYILRNQ DDREQWPRKF FNRTCKCTGN
101  FAGYNCGGCK FGWTGPDCNR KKPAILRRNI HSLTAQEREQ FLGALDLAKK
151  SIHPDYVITT QHWLGLLGPN GTQPQIANCS VYDFFVWLHY YSVRDTLLGP
201  GRPYKAIDFS HQGPAFVTWH RYHLLWLERE LQRLTGNESF ALPYWNFATG
251  KNECDVCTDD WLGAARQDDP TLISRNSRFS TWEIVCDSLD DYNRRVTLCN
301  GTYEGLLRRN KVGRNNEKLP TLKNVQDCLS LQKFDSPPFF QNSTFSFRNA
351  LEGFDKADGT LDSQVMNLHN LAHSFLNGTN ALPHSAANDP VFVVLHSFTD
401  AIFDEWLKRN NPSTDAWPQE LAPIGHNRMY NMVPFFPPVT NEELFLTAEQ
451  LGYNYAVDLS EEEAPVWSTT LGVVIGILGA FVLLLGLLAF LQYRRLRKGY
501  APLMETGLSS KRYTEEA
```

HUMAN TRP2

1	MSPLWWGFLL SCLGCKILPG AQGQFPRVCM TVDSLVNKEC CPRLGAESAN
51	VCGSQQGRGQ CTEVRADTRP WSGPYILRNQ DDRELWPRKF FHRTCKCTGN
101	FAGYNCGDCK FGWTGPNCER KKPPVIRQNI HSLSPQEREQ FLGALDLAKK
151	RVHPDYVITT QHWLGLLGPN GTQPQFANCS VYDFFVWLHY YSVRDTLLGP
201	GRPYRAIDFS HQGPAFVTWH RYHLLCLERD LQRLIGNESF ALPYWNFATG
251	RNECDVCTDQ LFGAARPDDP TLISRNSRFS SWETVCDSLD DYNHLVTLCN
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351	LEGFDKADGT LDSQVMSLHN LVHSFLNGTN ALPHSAANDP IFVVLHSFTD
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FIG. 4B

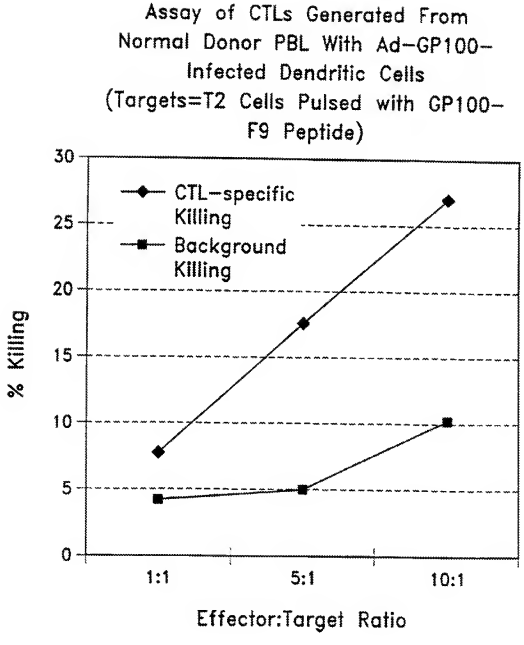
Assay of CTLs Generated From Normal Donor PBL With Ad-GP100-Infected Dendritic Cells (Targets=T2 Cells Pulsed with GP100-F9 Peptide)
CTL-specific Killing
Background Killing
% Killing
30
25
20
15
10
5
0
1:1
5:1
10:1
Effector:Target Ratio

FIG. 5

SEQUENCE LISTING

<110> Nicolette, Charles A.
Genzyme Corporation

<120> COMPOSITIONS AND METHODS FOR ANTIGEN-SPECIFIC VACCINATION

<130> 159792001040

<140> Unassigned
<141> 1999-03-19

<150> 60/078,890
<151> 1998-03-20

<160> 17

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Leu Gly Val Ser Arg Gln Leu Arg Thr Lys Ala Trp Asn Arg Gln Leu
        35                  40                  45

Tyr Pro Glu Trp Thr Glu Ala Gln Arg Leu Asp Cys Trp Arg Gly Gly
    50                  55                  60

Gln Val Ser Leu Lys Val Ser Asn Asp Gly Pro Thr Leu Ile Gly Ala
65                  70                  75                  80

Asn Ala Ser Phe Ser Ile Ala Leu Asn Phe Pro Gly Ser Gln Lys Val
                85                  90                  95

Leu Pro Asp Gly Gln Val Ile Trp Val Asn Asn Thr Ile Ile Asn Gly
            100                 105                 110

Ser Gln Val Trp Gly Gly Gln Pro Val Tyr Pro Gln Glu Thr Asp Asp
        115                 120                 125

Ala Cys Ile Phe Pro Asp Gly Gly Pro Cys Pro Ser Gly Ser Trp Ser
    130                 135                 140

Gln Lys Arg Ser Phe Val Tyr Val Trp Lys Thr Trp Gly Gln Tyr Trp
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Gln Val Leu Gly Gly Pro Val Ser Gly Leu Ser Ile Gly Thr Gly Arg
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Ala Met Leu Gly Thr His Thr Met Glu Val Thr Val Tyr His Arg Arg
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Gly Ser Arg Ser Tyr Val Pro Leu Ala His Ser Ser Ser Ala Phe Thr
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Ile Thr Asp Gln Val Pro Phe Ser Val Ser Val Ser Gln Leu Arg Ala
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Leu Asp Gly Gly Asn Lys His Phe Leu Arg Asn Gln Pro Leu Thr Phe
225                 230                 235                 240

Ala Leu Gln Leu His Asp Pro Ser Gly Tyr Leu Ala Glu Ala Asp Leu
                245                 250                 255

Ser Tyr Thr Trp Asp Phe Gly Asp Ser Ser Gly Thr Leu Ile Ser Arg
            260                 265                 270

Ala Leu Val Val Thr His Thr Tyr Leu Glu Pro Gly Pro Val Thr Ala
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Gln Val Val Leu Gln Ala Ala Ile Pro Leu Thr Ser Cys Gly Ser Ser
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Pro Val Pro Gly Thr Thr Asp Gly His Arg Pro Thr Ala Glu Ala Pro
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Asn Thr Thr Ala Gly Gln Val Pro Thr Thr Glu Val Val Gly Thr Thr
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Pro Gly Gln Ala Pro Thr Ala Glu Pro Ser Gly Thr Thr Ser Val Gln
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Ala Glu Ser Thr Gly Met Thr Pro Glu Lys Val Pro Val Ser Glu Val
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Met Gly Thr Thr Leu Ala Glu Met Ser Thr Pro Glu Ala Thr Gly Met
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Thr Pro Ala Glu Val Ser Ile Val Val Leu Ser Gly Thr Thr Ala Ala
                405                 410                 415

Gln Val Thr Thr Thr Glu Trp Val Glu Thr Thr Ala Arg Glu Leu Pro
            420                 425                 430

Ile Pro Glu Pro Glu Gly Pro Asp Ala Ser Ser Ile Met Ser Thr Glu
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Ser Ile Thr Gly Ser Leu Gly Pro Leu Leu Asp Gly Thr Ala Thr Leu
    450                 455                 460

Arg Leu Val Lys Arg Gln Val Pro Leu Asp Cys Val Leu Tyr Arg Tyr
465                 470                 475                 480

Gly Ser Phe Ser Val Thr Leu Asp Ile Val Gln Gly Ile Glu Ser Ala
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Glu Ile Leu Gln Ala Val Pro Ser Gly Glu Gly Asp Ala Phe Glu Leu
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Ser Ser Pro Gly Cys Gln Pro Pro Ala Gln Arg Leu Cys Gln Pro Val
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Leu Pro Ser Pro Ala Cys Gln Leu Val Leu His Gln Ile Leu Lys Gly
545                 550                 555                 560

Gly Ser Gly Thr Tyr Cys Leu Asn Val Ser Leu Ala Asp Thr Asn Ser
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Leu Ala Val Val Ser Thr Gln Leu Ile Met Pro Gly Gln Glu Ala Gly
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Leu Gly Gln Val Pro Leu Ile Val Gly Ile Leu Leu Val Leu Met Ala
        595                 600                 605

Val Val Leu Ala Ser Leu Ile Tyr Arg Arg Arg Leu Met Lys Gln Asp
    610                 615                 620

Phe Ser Val Pro Gln Leu Pro His Ser Ser Ser His Trp Leu Arg Leu
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Pro Arg Ile Phe Cys Ser Cys Pro Ile Gly Glu Asn Ser Pro Leu Leu
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acgaagtgga tacagaacct tgatggacaa aaggcgtcat attggtattc aaaaaacctc 180

aagggaaaga tgctcatgtg agagccctga tcaccaggac agccgactgt ctttctcaaga 240

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ctgctcatcg gctgttggta ttgtagaaga cgaaatggat acagagcctt gatggataaa 180

agtcttcatg ttggcactca atgtgcctta acaagaagat gcccacaaga agggtttgat 240

catcgggaca gcaaagtgtc tcttcaagag aaaaactgtg aacctgtggt tcccaatgct 300

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35 40 45

Asp Lys Arg Arg His Ile Gly Ile Gln Lys Thr Ser Arg Glu Arg Cys
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        35                  40                  45

Arg Arg Arg Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val
    50                  55                  60

Gly Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp
65                  70                  75                  80

His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val
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Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser
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          5                  10                  15

ggc cac tct tac acc acg gct gaa gag gcc gct ggg atc ggc atc ctg   152
Gly His Ser Tyr Thr Thr Ala Glu Glu Ala Ala Gly Ile Gly Ile Leu
     20                  25                  30

aca gtg atc ctg gga gtc tta ctg ctc atc ggc tgt tgg tat tgt aga   200
Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys Arg
 35                  40                  45

aga cga aat gga tac aga gcc ttg atg gat aaa agt ctt cat gtt ggc   248
Arg Arg Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val Gly
 50                  55                  60                  65

act caa tgt gcc tta aca aga aga tgc cca caa gaa ggg ttt gat cat   296
Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp His
                 70                  75                  80

cgg gac agc aaa gtg tct ctt caa gag aaa aac tgt gaa cct gtg gtt   344
Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val Val
             85                  90                  95

ccc aat gct cca cct gct tat gag aaa ctc tct gca gaa cag tca cca   392
Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser Pro
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100 105 110

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Pro Pro Tyr Ser Pro
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caggctggtc tcaaactcct gacctcaggt gatctgcccg cctcagcctc ccaaagtgct 1227

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Leu Thr Val Ile Leu Gly Val Leu Leu Leu Ile Gly Cys Trp Tyr Cys
35 40 45

Arg Arg Arg Asn Gly Tyr Arg Ala Leu Met Asp Lys Ser Leu His Val

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     50                  55                  60

Gly Thr Gln Cys Ala Leu Thr Arg Arg Cys Pro Gln Glu Gly Phe Asp
 65                  70                  75                  80

His Arg Asp Ser Lys Val Ser Leu Gln Glu Lys Asn Cys Glu Pro Val
                85                  90                  95

Val Pro Asn Ala Pro Pro Ala Tyr Glu Lys Leu Ser Ala Glu Gln Ser
            100                 105                 110

Pro Pro Pro Tyr Ser Pro
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            20                  25                  30

Asp Gly Val Leu Asn Lys Glu Cys Cys Pro Pro Leu Gly Pro Glu Ala
        35                  40                  45

Thr Asn Ile Cys Gly Phe Leu Glu Gly Arg Gly Gln Cys Ala Glu Val
    50                  55                  60

Gln Thr Asp Thr Arg Pro Trp Ser Gly Pro Tyr Ile Leu Arg Asn Gln
 65                  70                  75                  80

Asp Asp Arg Glu Gln Trp Pro Arg Lys Phe Phe Asn Arg Thr Cys Lys
                85                  90                  95

Cys Thr Gly Asn Phe Ala Gly Tyr Asn Cys Gly Gly Cys Lys Phe Gly
            100                 105                 110

Trp Thr Gly Pro Asp Cys Asn Arg Lys Lys Pro Ala Ile Leu Arg Arg
        115                 120                 125

Asn Ile His Ser Leu Thr Ala Gln Glu Arg Glu Gln Phe Leu Gly Ala
    130                 135                 140

Leu Asp Leu Ala Lys Lys Ser Ile His Pro Asp Tyr Val Ile Thr Thr
145                 150                 155                 160

Gln His Trp Leu Gly Leu Leu Gly Pro Asn Gly Thr Gln Pro Gln Ile
                165                 170                 175

Ala Asn Cys Ser Val Tyr Asp Phe Phe Val Trp Leu His Tyr Tyr Ser
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Val Arg Asp Thr Leu Leu Gly Pro Gly Arg Pro Tyr Lys Ala Ile Asp
        195                 200                 205

Phe Ser His Gln Gly Pro Ala Phe Val Thr Trp His Arg Tyr His Leu
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                245                 250                 255

Cys Thr Asp Asp Trp Leu Gly Ala Ala Arg Gln Asp Asp Pro Thr Leu
            260                 265                 270

Ile Ser Arg Asn Ser Arg Phe Ser Thr Trp Glu Ile Val Cys Asp Ser
        275                 280                 285

Leu Asp Asp Tyr Asn Arg Arg Val Thr Leu Cys Asn Gly Thr Tyr Glu
    290                 295                 300

Gly Leu Leu Arg Arg Asn Lys Val Gly Arg Asn Asn Glu Lys Leu Pro
305                 310                 315                 320

Thr Leu Lys Asn Val Gln Asp Cys Leu Ser Leu Gln Lys Phe Asp Ser
                325                 330                 335

Pro Pro Phe Phe Gln Asn Ser Thr Phe Ser Phe Arg Asn Ala Leu Glu
            340                 345                 350

Gly Phe Asp Lys Ala Asp Gly Thr Leu Asp Ser Gln Val Met Asn Leu
        355                 360                 365

His Asn Leu Ala His Ser Phe Leu Asn Gly Thr Asn Ala Leu Pro His
    370                 375                 380

Ser Ala Ala Asn Asp Pro Val Phe Val Val Leu His Ser Phe Thr Asp
385                 390                 395                 400

Ala Ile Phe Asp Glu Trp Leu Lys Arg Asn Asn Pro Ser Thr Asp Ala
                405                 410                 415

Trp Pro Gln Glu Leu Ala Pro Ile Gly His Asn Arg Met Tyr Asn Met
            420                 425                 430

Val Pro Phe Phe Pro Pro Val Thr Asn Glu Glu Leu Phe Leu Thr Ala
        435                 440                 445

Glu Gln Leu Gly Tyr Asn Tyr Ala Val Asp Leu Ser Glu Glu Glu Ala
    450                 455                 460

Pro Val Trp Ser Thr Thr Leu Ser Val Val Ile Gly Ile Leu Gly Ala
465                 470                 475                 480

Phe Val Leu Leu Leu Gly Leu Leu Ala Phe Leu Gln Tyr Arg Arg Leu
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Tyr Thr Glu Glu Ala
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Asp Ser Leu Val Asn Lys Glu Cys Cys Pro Arg Leu Gly Ala Glu Ser
        35                  40                  45

Ala Asn Val Cys Gly Ser Gln Gln Gly Arg Gly Gln Cys Thr Glu Val
    50                  55                  60

Arg Ala Asp Thr Arg Pro Trp Ser Gly Pro Tyr Ile Leu Arg Asn Gln
65                  70                  75                  80

Asp Asp Arg Glu Leu Trp Pro Arg Lys Phe Phe His Arg Thr Cys Lys
                85                  90                  95

Cys Thr Gly Asn Phe Ala Gly Tyr Asn Cys Gly Asp Cys Lys Phe Gly
            100                 105                 110

Trp Thr Gly Pro Asn Cys Glu Arg Lys Lys Pro Pro Val Ile Arg Gln
        115                 120                 125

Asn Ile His Ser Leu Ser Pro Gln Glu Arg Glu Gln Phe Leu Gly Ala
    130                 135                 140

Leu Asp Leu Ala Lys Lys Arg Val His Pro Asp Tyr Val Ile Thr Thr
145                 150                 155                 160

Gln His Trp Leu Gly Leu Leu Gly Pro Asn Gly Thr Gln Pro Gln Phe
                165                 170                 175

Ala Asn Cys Ser Val Tyr Asp Phe Phe Val Trp Leu His Tyr Tyr Ser
            180                 185                 190

Val Arg Asp Thr Leu Leu Gly Pro Gly Arg Pro Tyr Arg Ala Ile Asp
        195                 200                 205

Phe Ser His Gln Gly Pro Ala Phe Val Thr Trp His Arg Tyr His Leu
    210                 215                 220

Leu Cys Leu Glu Arg Asp Leu Gln Arg Leu Ile Gly Asn Glu Ser Phe
225                 230                 235                 240

Ala Leu Pro Tyr Trp Asn Phe Ala Thr Gly Arg Asn Glu Cys Asp Val
                245                 250                 255

Cys Thr Asp Gln Leu Phe Gly Ala Ala Arg Pro Asp Asp Pro Thr Leu
            260                 265                 270

Ile Ser Arg Asn Ser Arg Phe Ser Ser Trp Glu Thr Val Cys Asp Ser
        275                 280                 285

Leu Asp Asp Tyr Asn His Leu Val Thr Leu Cys Asn Gly Thr Tyr Glu
    290                 295                 300
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Gly Leu Leu Arg Arg Asn Gln Met Gly Arg Asn Ser Met Lys Leu Pro
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Thr Leu Lys Asp Ile Arg Asp Cys Leu Ser Leu Gln Lys Phe Asp Asn
                325                 330                 335

Pro Pro Phe Phe Gln Asn Ser Thr Phe Ser Phe Arg Asn Ala Leu Glu
            340                 345                 350

Gly Phe Asp Lys Ala Asp Gly Thr Leu Asp Ser Gln Val Met Ser Leu
        355                 360                 365

His Asn Leu Val His Ser Phe Leu Asn Gly Thr Asn Ala Leu Pro His
    370                 375                 380

Ser Ala Ala Asn Asp Pro Ile Phe Val Val Leu His Ser Phe Thr Asp
385                 390                 395                 400

Ala Ile Phe Asp Glu Trp Met Lys Arg Phe Asn Pro Pro Ala Asp Ala
                405                 410                 415

Trp Pro Gln Glu Leu Ala Pro Ile Gly His Asn Arg Met Tyr Asn Met
            420                 425                 430

Val Pro Phe Phe Pro Pro Val Thr Asn Glu Glu Leu Phe Leu Thr Ser
        435                 440                 445

Asp Gln Leu Gly Tyr Ser Tyr Ala Ile Asp Leu Pro Val Ser Val Glu
    450                 455                 460

Glu Thr Pro Gly Trp Pro Thr Thr Leu Leu Val Val Met Gly Thr Leu
465                 470                 475                 480

Val Ala Leu Val Gly Leu Phe Val Leu Leu Ala Phe Leu Gln Tyr Arg
                485                 490                 495

Arg Leu Arg Lys Gly Tyr Thr Pro Leu Met Glu Thr His Leu Ser Ser
            500                 505                 510

Lys Arg Tyr Thr Glu Glu Ala
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/06034

A. CLASSIFICATION OF SUBJECT MATTER
IPC(6) :Please See Extra Sheet.
US CL :Please See Extra Sheet.
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/184.1, 277.1, 287.1, 93.2, 93.71; 435/372, 373; 514/2, 44; 530/350, 806, 827, 828; 536/23.1, 23.5

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

DIALOG medicine index, APS, WEST

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A,P	LIU, M. Transfected human dendritic cells as cancer vaccines. Nat. Biotech. April 1988, Vol. 16, pages 335-336, see entire document.	1-23
A	DONNELLY, J.J. et al. DNA vaccines. Ann. Rev. Immunol. 1997, Vol. 15, pages 617-648, see entire document.	1-23
A, P	PARDOLL, D.M. Cancer vaccines. Nature Medicine. May 1998, Vol. 4, No.5(suppl.), pages 525-531, see entire document.	1-23

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 JUNE 1999	03 AUG 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	Authorized officer F PIERRE VANDERVEGT
Facsimile No. (703) 305-3230	Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/06034

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOCZKOWSKI, D. et al. Dendritic cells pulsed with RNA are potent antigen-presenting cells in vitro and in vivo. J. Exp. Med. 01 August 1996, Vol. 184, No. 2, pages 465-472, see entire document, abstract in particular.	1-3, 7-10, 14-17, 20, 22
X	US 5,679,647 A (CARSON et al) 21 October 1997, see entire document.	1-3, 13, 20-23
X, P ---- Y, P	US 5,844,075 A (KAWAKAMI et al) 01 December 1998, see entire document.	1, 13, 20, 22-23 ---- 2-12, 14-19, 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/06034

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A01N 37/18, 43/04, 63/00; A61K 31/70, 35/12, 35/36, 39/00; C12N 5/02, 5/06; C07K 1/00; C07H 21/02, 21/04

A. CLASSIFICATION OF SUBJECT MATTER:
US CL :

424/184.1, 277.1, 287.1, 93.2, 93.71; 435/372, 373; 514/2, 44; 530/350, 806, 827, 828; 536/23.1, 23.5